

# EVALUATION OF A MECHANICAL COTTONSEED DELINTER FOR BREEDERS

A Thesis

by

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## ABSTRACT

Delinting cottonseed, which is removing short fuzz fiber called linters to polish seed for mechanical planting, is a practice commonly used by cotton breeding programs. The predominant method of delinting cottonseed is acid delinting, which can be dangerous and produces toxic effluent. Disposal of this effluent is costly. Current research on mechanical delinting proposes an alternative to acid delinting. A prototype for commercial delinting developed by USDA-ARS Cotton Production and Processing Research Unit in Lubbock, TX, was used to explore advantages and disadvantages of using a mechanical delinter for small breeder samples. Delinting time, seed carryover between samples, incidence of seed-borne disease, seed size effects and sample size effects were evaluated and compared to acid delinting. Different cantilever brush configurations were tested for efficiencies by processing separate small samples. Seed quality and germination for mechanical and acid delinted samples was compared. Modifications to the cantilever brush system and to the drum decreased delinting time and increased ease of sample processing compared to the original prototype. Small improvements in reduction of seed carryover and in seed-borne disease incidence were observed, but these areas still need improvement. Mechanically delinted seed averaged 87 percent germination using a wet towel method compared to 89 percent for acid delinted seed in 2016. In 2017, mechanically delinted seed planted in a field environment averaged 85 percent germination when a packed drum treatment was used and 76 percent germination when a finished drum treatment was used.

## DEDICATION

I would like to dedicate this thesis to my amazing parents for raising me to become the person that I am today and encouraging me through all these years of college.

Thanks to my wife and children for sticking by me through the long hours and the tough times.

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Thanks, is also deserving for the members of the Cotton Breeding Program in Lubbock for assisting me throughout the entirety of my research. The work they did was sometimes tedious and seemed unimportant but without their help it would have been more daunting and time consuming. And finally, thanks to my mother and father for their encouragement and to my wife and children for their patience, love, support, and encouragement.

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## NOMENCLATURE

°C	Degrees Celsius
CaCO <sub>3</sub>	Calcium Carbonate
CFM	Cubic Feet per Minute
cm	centimeter
g	grams
in	inch
L	Liter
LREC	Lubbock Research and Extension Center
m	meter
MgSO <sub>4</sub>	Magnesium Sulfate
mL	milliliter
oz	ounce
SI	Seed Index; weight in grams of 100 fuzzy seed
USDA-ARS-CPPRU	United States Department of Agriculture-Agriculture Research Service- Crop Production and Processing Research Unit

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## 1. INTRODUCTION

The United States Department of Agriculture-Agricultural Research Service-Crop Production and Processing Research Unit (USDA-ARS-CPPRU) and Cotton Incorporated have investigated the potential of commercial mechanical cottonseed delinting. Safety and chemical disposal concerns led to development of a prototype mechanical delinter for small breeder samples.

Previous attempts at mechanical delinting affected seed quality by producing excessive heat resulting in seed coat damage (Olivier, 2005). The primary goal of these efforts has been to develop techniques to remove linters without adversely affecting germination. Earlier versions of the mechanical delinting system required up to ten minutes to process a single sample.

In an effort to increase efficiency, researchers at the USDA-ARS-CPPRU designed different cylindrical brushes to equip on the delinter to see if the delinting time could be reduced. A major concern for breeders is seed carry-over or mixing of seeds from one sample to the next. Two different drum treatments were suggested by USDA-ARS-CPPRU to determine if seed carry-over could be reduced. After testing had begun, seed-borne disease was identified as a possible problem since acid delinting can reduce some forms of seed-borne disease. Presence of seed-borne disease before and after mechanical delinting compared to acid delinting was added to the original objectives and investigated. Cotton breeders routinely advance and maintain multiple genotypes within their programs, so an experiment was conducted to investigate if mechanical delinting times varied between genotypes, especially those with different seed size.

## 2. LITERATURE REVIEW

### 2.1 Delinting

After ginning, cottonseed retains some short fibers on the seed coat, called linters. The removal of the short fibers is necessary so that planting seed will feed unimpeded through modern planting equipment. If linters are not removed, the linters will cause seed to clump together preventing the singulated flow of seed (Olivier, 2005). The process of removing linters is called delinting. The three main types of delinting are flame delinting, acid delinting, and mechanical delinting.

#### 2.1.1 Flame Delinting

The flame delinting method involves coating the fuzzy cottonseed with kerosene, heating the seed, and then flaming the seed. (USDA AMS, 2014). Flame delinting can be quite effective but is difficult to perform. Flamed seed has to be rapidly cooled and if done improperly damage may incur resulting in poor germination and a poor stand. Though flame delinting greatly improves flowability of seed, results do not meet the standards for modern precision planters (Delouche, 1986). Flame delinting is no longer used due to the substandard control of treated seed quality (Delouche, 1981).

#### 2.1.2 Acid Delinting

Use of acid delinting was first reported in 1911 (Duggar and Cauthen, 1911). At the time it was being used to control seed borne disease (Christidis and Harrison, 1955). Acid delinting is effective in preventing seed transmission of bacterial blight, *Xanthamonas campestris* pv *malvacearum*, and anthracnose, *Glomerella gossypii* or *Colletotrichum gossypii* var. *cephalosporioides* (Brown, 1969; Savoy, 1995; Drummond and Savoy, 1996). Today, acid is the most prevalent type of delinting used in planting seed production. Acid delinting can be

performed using concentrated sulfuric acid, dilute sulfuric acid or hydrochloric acid gas. The processes for the different types of acid delinting are essentially the same. Seed is thoroughly coated with acid which then chemically burns off the linters. The seed is then rubbed to remove any remnants of the burned linters. Finally, the seed is neutralized to remove any remaining residual acid (Gregg and Billups, 2010). Concentrated sulfuric acid delinting is primarily used for small volumes of seed usually of less than 40 Kg and is the most widely used method for cotton breeding programs. It has drawbacks, including potential hazard to employees handling the concentrated acid and effluent produced from the delinting process (USDA AMS, 2014; Sharma, 2014).

### 2.1.3 Mechanical Delinting

The first patents issued for the creation of a mechanical delinter date back to the late 1890's with several patents issued in the following decades. The first patents issued were for machines that removed the linters using a mechanism that closely resembles the modern saw type mechanical delinter. There are basically two different methods of mechanically delinting cottonseed, saw delinting and brush delinting. Saw delinting is the preferred means of mechanically delinting large quantities of cottonseed. Saw delinting leaves approximately three to four percent of fibers on the seed (Sharma, 2014). Mechanical saw delinting has been shown to increase seed damage thereby decreasing overall seed quality (Delouche, 1986). Mechanical brush delinters only partially remove linters and planting seed requires further processing using small quantities of acid or polishing the seed for longer periods of time (Sharma, 2014). Mechanical delinting also can be used to process seed for oil production and delivers a higher oil quality after crushing than undelinted seed. The linters removed by delinting provide added



value to the cotton processors. Visitors from Georgia-Pacific have showed interest in the removed linters for the production of some potential products that could be developed.

## 2.2 Singulated Cottonseed Flow

Any linters remaining on planting seed can cause problems during planting. For farm production cottonseed, singulated seed is needed to flow through the planter unimpeded by linters. Currently, mechanically delinted cottonseed is not widely used for planting seed. One reason why mechanical delinters are not used for planting is due to the presence of linters which remain on the top and bottom of the seed. New designs of mechanical brush delinters remove a greater portion of linters and allow for singulation of cottonseed to be achieved without the need for additional treatment by acid delinting. Another technology was developed to achieve singulated flow of cottonseed. Olivier (2005), used a system called the Easi-flo system, in which a starch polymer coat was applied to cottonseed that was delinted using a mechanical saw delinter. In one test, polymer coated seed was compared to conventional acid delinted seed using commercial seed from the same lot for both treatments. There was no significant difference in emergence between the two treatments. The starch polymer coated seed was also tested using different coating levels. It was concluded that a coating rate above eight percent would have a significantly negative effect on germination rates (Olivier, 2005).

## 2.3 Fiber-Seed Attachment Force

Bechere, Zeng, and Hardin (2016) ran an experiment to investigate seed attachment force. Seed attachment force affects ginning productivity. Thirteen different cultivars were planted in four different environments. A one hundred boll sample was taken from each plot and ginned. Data on net ginning energy requirements, ginning rate, fuzz percent, lint percent, lint yield, and high-volume instrument (HVI) fiber quality traits were collected. The experiment

found significant differences between cultivars, net ginning energy, and ginning rate (Bechere, Zeng, Hardin, 2016). Since the ginning rates of the cultivars were different, it can be expected that mechanical delinting rates will differ as well. This hypothesis would have to be investigated further to reach a conclusion.

## 2.4 Mechanical Brush Delinter for Breeders

During the development of the mechanical delinter for breeders a series of experiments were conducted to determine the most effective design and operational factors. Researchers at the USDA-ARS-CPPRU in Lubbock, Texas, tested six different abrasive drum linings as well as a bare drum without a lining (Holt et al., 2017). The designation and descriptions of the drum linings that were used can be found in Table 1.

Table 1. Drum linings used for the USDA-ARS-CPPRU mechanical cottonseed delinting experiment and the designations and description including dimensions and brush types.

Lining	Designation	Description
3M Purple ScotchBrite™	PurpleSB	
3M Clean & Strip™	ClStrip	
3M Brushlon™	Brushlon 80	80 Grit
Nylon Brush	CarolinaB	Nylon brush with 0.10 cm diameter crimped bristle, 3.81cm bristle height
Nylon Brush & Wire Brush (72 Nylon & 12 steel wire)	CBw12wb	3.81cm bristle height; wire was crimped, 0.03 cm diameter with a 3.81 cm bristle height
42 Nylon & 42 steel wire	42N42W	Nylon brush with 0.10 cm diameter crimped bristle, 3.81cm bristle height; wire was crimped, 0.03 cm diameter with a 3.81 cm bristle height
Drum without abrasive material	None	Drum dimensions;30.48 cm inside-diameter and 20.3 cm wide

Along with the drum lining, the experiment used one or two roller brushes. The roller brushes were constructed with ten wire wheel brushes bored out to fit on a 2.54 cm shaft with a 0.32 cm keyway. The wire wheel brushes had a 0.03 cm diameter and a wheel diameter of 10.16 cm and

bristle length of 1.75 cm. The testing included all combinations of drum linings and number of roller brushes at two different time intervals. Time intervals consisted of five-minute and ten-minute delinting times. Each combination was replicated three times using 340 g samples.

After the samples were delinted, percent lint loss, and the percent of visual mechanical damage were calculated as well as a germination test for the delinted samples. From the data that were collected it was determined that the combination of two roller brushes and the drum lining 42N42W outperformed the other combinations. The mixture of the wire and nylon brushes dissipated heat in comparison to a one hundred percent wire bristled brush. The one hundred percent wire bristled brush generated heat that could negatively impact germination. Other materials performed well but were deemed unsuitable because of lack of durability or difficulty during cleaning (Holt et al., 2017).

## 2.5 Other Uses and By-products

Mechanically delinted cottonseed is widely used by the dairy industry. Dairies use the partially delinted cottonseed from mechanical delinters to increase the flowability of the cottonseed delivered to cattle in automatic feeders. This feed seed retains some linters which provide added nutrients for the dairy cows (Morelra, et al., 2004). Linters removed by mechanical delinting can be used for a variety of products. These include high quality paper, absorbent cottons, cellulose acetate for plastics, cellulose ethers and esters for use in other products, cellulose nitrate for the manufacturing of accelerants, felts for many different products for upholstery, and low-quality yarn for products like twine and mop heads (Sharma, 2014).

### 3. MATERIALS AND METHODS

#### 3.1 Obtaining Seed for Testing

The seed used for testing was collected from cotton in border rows surrounding furrow irrigated tests at the Texas A&M AgriLife Research and Extension Center in Lubbock (LREC) in 2015. The field was managed according to standard recommendations for supplemental furrow irrigated cotton production in the Southern High Plains. The cotton was harvested using a modified John Deere JD 482 plot stripper.

The harvested cotton was ginned using a research laboratory gin equipped with a stage 1 Murray Separator and Murray Separator Vacuum. The samples then traveled through a Lummus 700 II Feeder Extractor and ginned on a Continental Eagle 10 saw gin stand with 10 in saws. The fiber was cleaned using a Moss Lint Cleaner and condensed with a Moss Lint Cleaner Condenser. The air was generated by a Phelps Model 25-K fan and a Smith Model 35 Fan. After the cotton was ginned, the seed was found to have an average seed index of 9.2. Seed index is the weight of 100 non-delinted seed in grams. This seed was used for the Optimum Timing, Timed-Sample Size, Seed Carry-Over, and Cantilever Configuration experiments. These experiments used clean seed meaning excessive trash was removed by picking out any large sticks and burrs by hand.

#### 3.2 Operating the Delinter

The mechanical delinter must be plugged into an electrical outlet and have a source for compressed air. An Ingersoll Rand® Garage Mate Portable Electric Air Compressor 2 HP, 20-Gallon Vertical, 5.5 CFM was connected to the delinter using a Goodyear® air hose to supply the air. The delinter uses the compressed air to remove delinted seed from the interior of the drum.

The delinter is equipped with multiple switches (Figure 1) located on the front left side to control the processes.

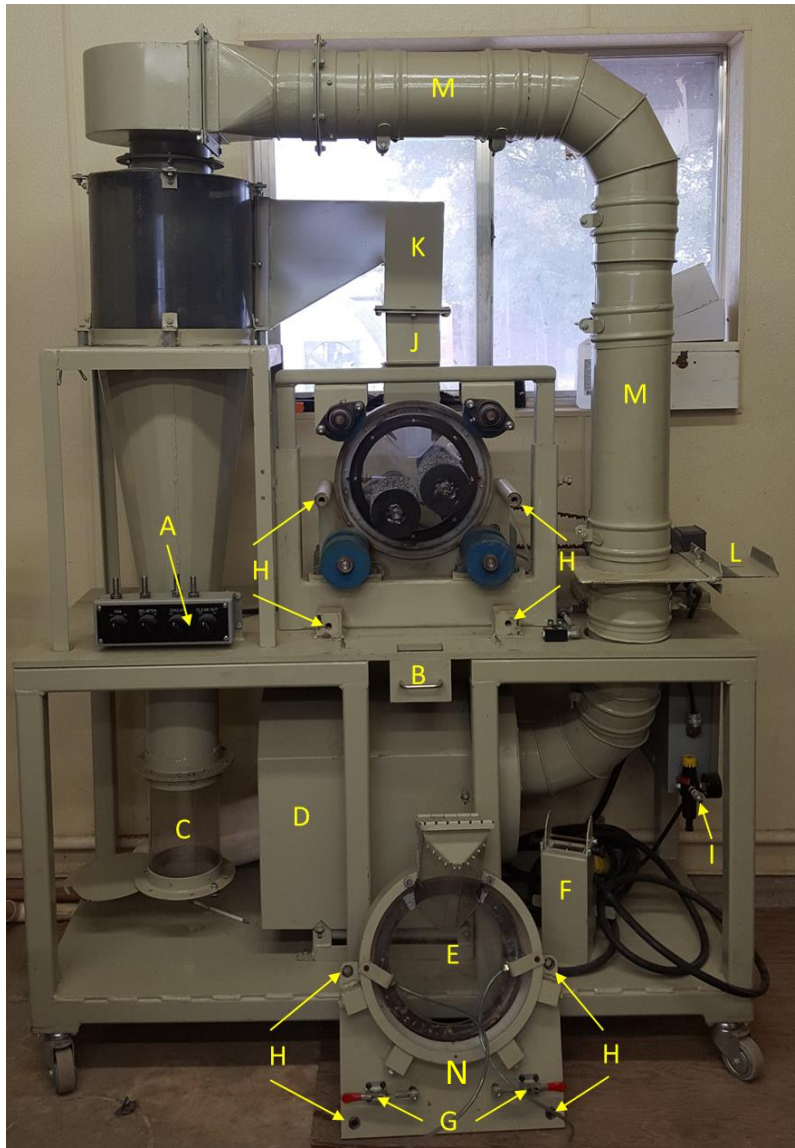


Figure 1. Image of mechanical cottonseed delinter used for testing and key Parts. A.) Control Panel; B.) Seed Collection Drawer; C.) Linter Collection Chamber; D.) Fan; E.) Front Face-Plate; F.) Seed Collection Door; G.) Seed Collection Door Clamps; H.) Front Face-Plate Bolt Holes; I.) Compressed Air Connection; J.) Linter Shoot; K.) Linter Removal Duct; L.) Air Slide M.) Air Duct; N.) Cottonseed Loading Slide.



Figure 2. Close-up image of mechanical cottonseed delinter control panel.

We followed a specific set of steps when operating the mechanical delinting device. First the fan is switched on and allowed to reach full speed, then the delinter is switched on and allowed to reach full speed (Figure 2). Caution should be used when plugging in the mechanical delinting equipment to ensure that an electrical breaker is not overloaded. After the seed has been delinted, the seed collection door, Figure 1.F, is manually opened. After the seed door is open, the clean-out switch on the control panel (Figure 2) is turned to use compressed air to blow-out any remaining seed from the delinter and into the seed collection drawer. Figure 1 illustrates the individual components of the delinter. The control panel (Figure 1.A) is used to control power to the mechanical delinter. Delinted cottonseed is removed from the drum and collected in the seed collection drawer (Figure 1.B). The linters removed from the fuzzy cottonseed are contained in the linter collection chamber for removal (Figure 1.C). Air for linter removal is supplied by a fan located under the mechanical delinter table surface (Figure 1.D).

The front face plate is placed in front of the drum to complete the delinting chamber (Figure 1.E). The seed collection door is opened once delinting is complete to retrieve delinted seed (Figure 1.F). The seed collection door clamps secure the seed collection door to the front face-plate (Figure 1.G). The front face plate is attached to the delinter by bolts and the holes are aligned and fastened to secure the whole delinting chamber (Figure 1.H). Compressed air used to clean out the delinting chamber is supplied by a fitting located under the mechanical delinting table surface (Figure 1.I). The linter removal duct allows the linters to escape into the linter containment chamber (Figure 1.K). The air slide is the primary means for air handling control (Figure 1.L) with fine adjustments controlled by the linter shoot (Figure 1.J). Air from the fan is carried to the delinting chamber by an air duct (Figure 1.M). Seed inserter into the delinter using the cottonseed loading shoot (Figure 1.N).

### 3.3 Disassembling, Reassembling, and Cleaning the Delinter

#### 3.3.1 Disassembling the Delinter

The drum is removed by first removing the seed collection door (Figure 1.F) by releasing the two red-handled clamps (Figure 1.N). The front face-plate (Figure 1.E) is removed by unbolting the face plate using an electric impact wrench with a ½ in (12.7 mm) drive and a ¾ in (19.05 mm) impact socket.



Figure 3. Image of mechanical cottonseed delinter stock drum.

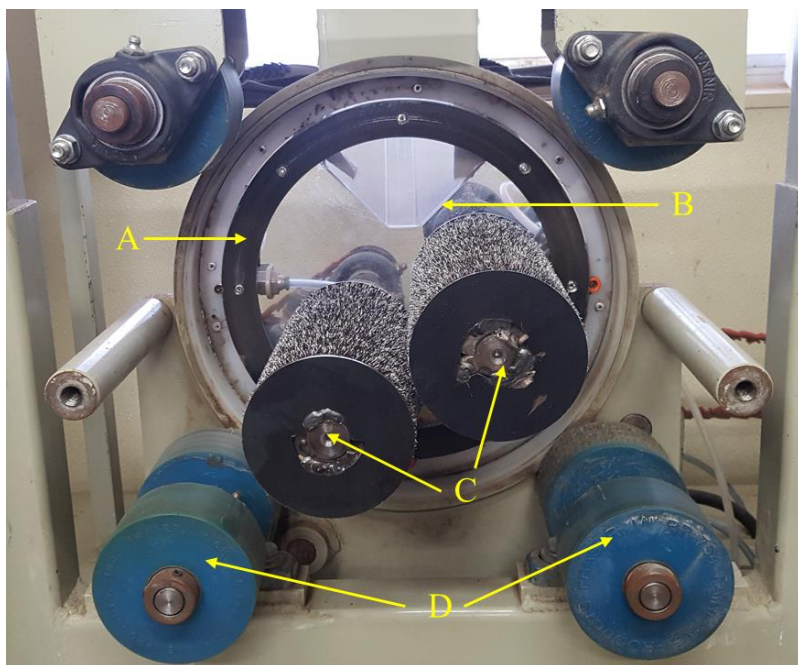


Figure 4. Close-up image of mechanical cottonseed delinting chamber with individual components labels. A.) Metal ring on back face-plate; B.) Back face-plate; C.) Cantilever brushes; D.) Drum rollers.



After the front face-plate has been removed, the drum in Figure 3 is removed by pulling and turning the drum until the drum is completely off the drum rollers (Figure 4.D).

### 3.3.2 Cleaning the Drum

The drum is cleaned by first picking out any remaining seeds or seed coat fragments with a 90-degree angle pick. Next, the drum is scrubbed along the longitude brushes with a standard wire brush. Compressed air is then used to remove remaining linters and fine trash.

### 3.3.3 Cleaning the Delinter

The delinter accumulates excess trash in the linter removal duct (Figure 1.L). Trash is removed by inserting a compressed air gun and blowing the trash into the linter collection chamber (Figure 1.C). The linter collection chamber is then opened, and the trash removed. The metal ring around the back-face plate will accumulate trash during operation, which is removed by blowing compressed air in between the metal ring and the back face-plate (Figure 4.A and 4.B).

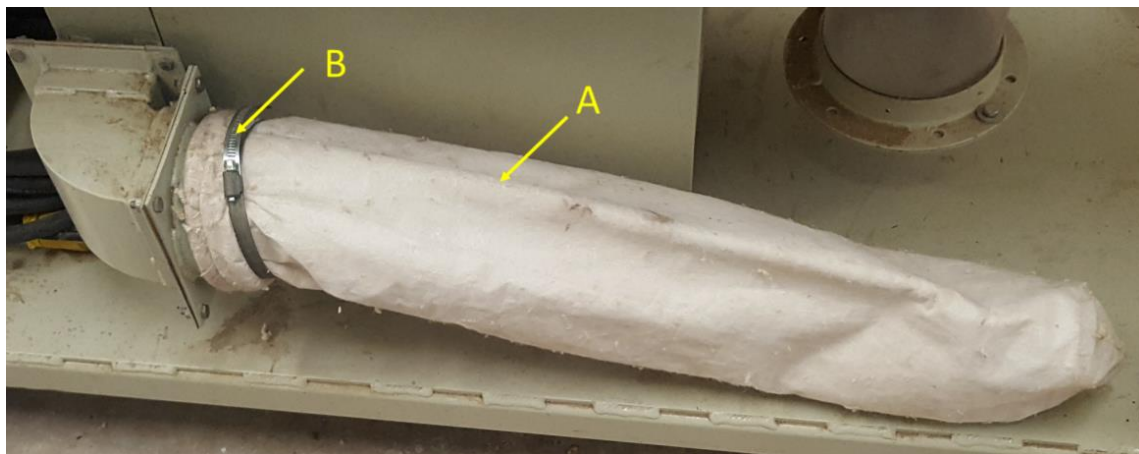


Figure 5. Image of the mechanical cottonseed delinter air filter connection. Air Filter (A) and Air Filter Hose Clamp (B).

Once the front face-plate has been removed, compressed air is used to blow any trash off the delinting frame. The fan air filter (Figure 5.A) is cleaned by removing the hose clamp (Figure 5.B) from the delinter, turning it inside out, brushing the linters off the filter with a wire brush, then using compressed air to remove any remaining dust. The delinter table surface is cleaned by blowing the table surface with compressed air.

#### 3.3.4 Reassembling the Delinter

After the drum has been cleaned, the drum is replaced by setting it on the drum rollers (Figure 4.D). Once on the drum rollers, the drum is pushed while rolling until seated on the back-face-plate (Figure 4.B). The front face-plate is then reattached by lining up the face-plate with the bolt holes (Figure 2.H) then inserting the bolts with lock washers and tightening. The seed collection door (Figure 2.F) is reattached by aligning the door to the front face-plate (Figure 2.E) and clamping the seed collection door clamps (Figure 2.G) to the front face-plate (Figure 2.E.). The fan air filter (Figure 5.A) is replaced and the hose clamp (Figure 5.B) tightened.

#### 3.4 Optimum Timing Experiment

The optimum timing experiment was conducted to explore the optimum time required to delint samples in a range of various sizes (based on weight). Nine sample sizes were tested, beginning at 50 g and increasing in 50 g increments to a maximum of 450 g. The experiment was deployed as an RCBD with four replications and each of the nine sample sizes were represented in all replications. The delinter was cleaned out between each replication to remove variation due to linter build-up on brushes. Samples were delinted until all linters visually appeared to be removed and the time to delint was recorded. The null hypothesis stated that delinting time would not differ between samples of different weights. After the delinting times were recorded from the experiment, data were analyzed using a least squares model and a means

comparison using JMP 12 Pro. Tables were generated by exporting the JMP 12 Pro reports into Microsoft Excel. The figures also were generated using Microsoft Excel.

Based on the results of the cantilever configuration brush experiment, a second optimum timing experiment was conducted. The results of the cantilever configuration experiment suggested that configuration 16 (both cantilever brushes with a stacked design) was the most efficient configuration. The objective of this second timing experiment was to determine the optimum delinting time of various sample sizes using this configuration. The randomization and delinting method remained the same as the first optimum timing experiment, but the original seed source was exhausted. Excess seed from the cantilever brush configuration experiment was used. Data was analyzed the same as the original optimum timing experiment.

An additional objective addressed during this experiment was the effect of mechanical delinting on germination rates. This was addressed by comparing the germination rates of mechanically delinted seed versus acid delinted seed. Seed from the timed experiment was used for these germination tests. The germination test was performed by moistening a germination towel and placing 20 seed from each sample on a towel. The first towel was then covered with another moist towel and rolled. Once all the samples were placed on towels and rolled, they were then placed into a plastic tote and covered with its matching lid. The plastic totes were then placed inside an Enconair Plant Growth Chamber, Model SG-30 set on 30 °C with a relative humidity of 85% with lighting alternating every 16 hours. The towels were removed after four days, the number of seeds germinated per sample were counted and a germination percentage was calculated.

### 3.5 Seed Carry-Over Experiment

The effect of drum design on seed carry-over was examined to determine the effectiveness of three different drum modifications: unaltered or stock, cotton packed, and finished.

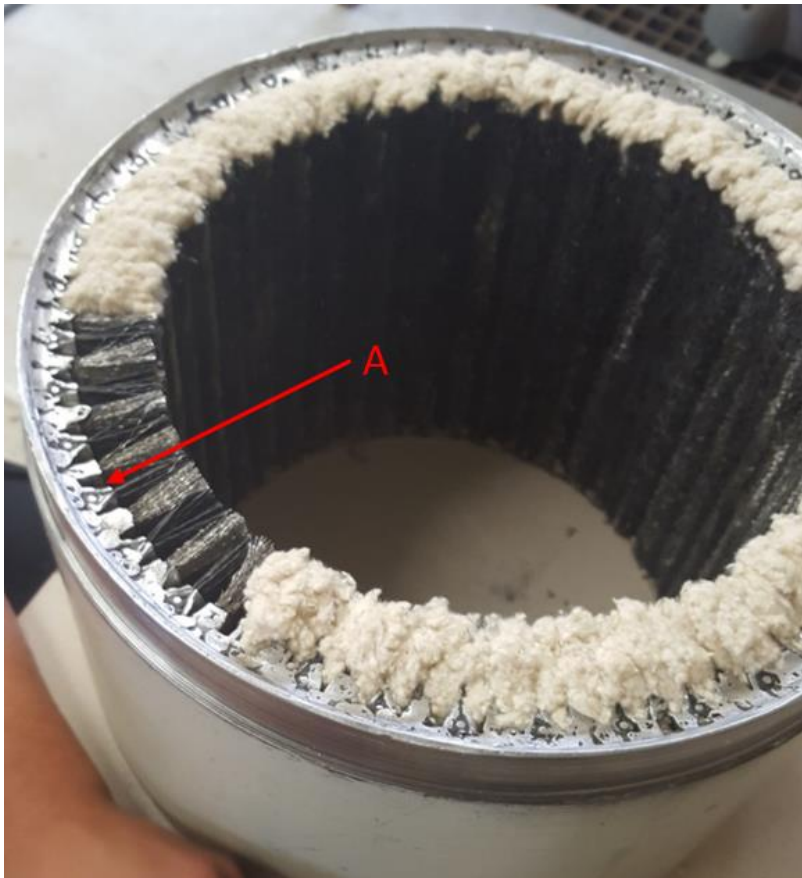


Figure 6. Image of a packed drum on mechanical cottonseed delinter with voids between brushes and the voids filled with cotton lint (A). Voids

The packed drum treatment, Figure 6, used a drum with cotton lint placed in the voids (Figure 6.A) between the brushes inside the drum. The drum was packed by taking a portion of cotton lint, hand rolling into a conical shape, starting insertion by placing the tip of the cone into the void then finishing insertion by pushing into the void with a thin screwdriver. This was done for every void. The drum was cleaned after each replication and then repacked.



Figure 7. A finished drum coated with 3M™ Fire-Barrier Sealant Caulk CP 25WB injected into the voids in the drum and 3M Fire-Block Sealant FB136 laid over the 3M™ Fire-Barrier Sealant Caulk CP 25WB.

The finished drum treatment used a drum sealed with two types of fire-proofing materials (Figure 7). First, a 3M™ Fire-Barrier Sealant Caulk CP 25WB was injected into the voids using a caulking gun and the sealant left to dry according to the label. Next, 3M Fire-Block Sealant FB136 was laid over the 3M Fire-Barrier Sealant Caulk CP 25WB from the outer circumference of the drum to the tips of the brushes inside the drum so that the voids were completely covered.

A single drum was used and modified as the experiment progressed to eliminate the possibility that using three different drums would add unintended variation. This necessitated that four replications be run at each stage of the drum modification.

Eighty-gram samples of a super-okra leaf cotton variety and a normal leaf cotton variety were used in the test. The okra leaf cotton was used as a phenotypic marker to determine carryover. First, an okra leaf cottonseed sample was delinted, then four separate normal leaf cottonseed samples of the same variety were delinted, followed by a complete cleaning of the drum. This was repeated four times, for each progressive modification of the drum. All delinted seeds from the normal leaf samples were saved for the next step of the experiment, planting of seed, and observation of plants. The objective of this approach was to identify the amount of okra leaf plants that were a result of seed carrying over into the normal leaf samples during delinting.

During the first year of the experiment (2016), the normal drum treatment samples were planted in 32 oz Styrofoam cups in the greenhouse complex at the LREC. The cups were planted with 6 seeds per cup and left to grow in the greenhouse. While the samples were being planted, the total number of seeds were counted for each delinted sample. Once the plants had reached the six true leaf stage, the assessment of okra leaf plant contamination was made.

Due to the amount of time and space the experiment required in the greenhouses in 2016, seed samples processed with the pack and finished drum were planted in a field at the LREC in 2017 for observation and plant counts. The delinted samples were planted in 4-row plots until seed was exhausted with 1.22 m (4 ft) alleys planted in between to distinguish between plots. An okra leaf plant count was then taken at the six true leaf stage. The experiment was repeated in

2018 using the top performing finished drum and the best performing brush combination from the cantilever configuration experiment.

A null hypothesis was developed to investigate seed carry-over between samples. The null hypothesis stated that seed carry-over would not occur. After the data from the experiment was recorded and analyzed using JMP 12 Pro, tables were generated with Microsoft Excel 2010. The drum treatment used in 2016 was the original drum design, and data from 2016 was analyzed separately due to being planted inside a greenhouse environment. In addition to the seed carry-over experiment, a field germination rate was taken by estimating the number of seeds planted. The estimated number of seeds was calculated by taking a seed index of delinted seed and dividing the total weight of the delinted cottonseed sample by the seed index and multiplying by 100. Stand counts were taken to see how many plants came up in each row and calculating the average germination.

### 3.6 Seed Borne Disease Experiment

*Xanthamonas citri* subsp. *malvacearum* (Xcm) is a seed borne bacteria that infects cotton. It is less prevalent with acid delinting due to the likelihood that the acid kills bacteria on the surface of the seed coat. The bacteria located internally in seed can survive acid delinting. To determine if mechanically delinting cotton prevents seed transmission of Xcm, a series of tests were run. First, cottonseed harvested from a Xcm infected field was subsampled and one subsample was acid delinted and one subsample was mechanically delinted using a stock delinter drum.

To transfer bacteria from the delinted seed subsamples, a potato carrot dextrose agar medium was made. The medium was produced by filling a 1 L Erlenmeyer flask with 800 mL of distilled water. Next, the flask filled with distilled water was placed on a stir plate and 0.3 g of

MgSO<sub>4</sub> was added along with 0.2 g of CaCO<sub>3</sub>, 10 g of agar, 40 g of potato dextrose agar, 2.5 g of peptone, 0.5 g yeast extract, and 15 mL of carrot juice. The flask was then filled with distilled water until the mixture was 1000 mL. The potato carrot dextrose agar mixture was autoclaved to remove any biologic impurities. The mixture was then poured into petri dishes and left to cool to room temperature. To decrease fungal growth, seed were treated with a fungicidal treatment. This fungicidal treatment included 5.4 mL of EverGol Prime, 12.7 mL of Allegiance FL, 2.5 mL of Proline, and 700mL of distilled water. When the fungicidal treatment was produced, it was placed on a stir plate to keep the chemicals suspended in a solution. A portion of the fungicidal solution was placed in a bowl on another stir plate. Subsamples were placed in a strainer and dipped into the bowl with the fungicidal solution. Once all the seeds were thoroughly coated, the sample was removed from the strainer and left to dry.

A hundred treated seed from each delinting method were plated with ten seeds per plate on the petri dishes. The bacteria were left to grow and yellow colored bacteria colonies consistent with Xcm were isolated by scooping onto a ring probe which had been sterilized with heat using an alcohol lamp. The bacteria were then transferred to clean potato dextrose plates. To confirm the presence of Xcm from the clean cultures, samples from these cultures were applied to resistant and susceptible varieties of plants for validation. This was accomplished by planting the resistant and susceptible cotton varieties in Ray Leach SC10 Cone-tainers<sup>™</sup> (Stuewe & Sons, Inc., Tangent, OR) with potting soil and placing them into a growth chamber to await inoculation. Each variety was represented by 88 plants. The plants were left to germinate and emerge. At the cotyledon stage, plants were inoculated with the pure bacteria samples by collecting a piece of bacterial mass on an autoclaved toothpick and scratching a large X on the underside of both cotyledons taking care not to puncture all the way through the



cotyledons. The plants were placed in the dark in a humidity chamber at 100% humidity for 24 hours. Plants were then returned to an incubation chamber (27 °C) to continue to grow. After two weeks, the plants were rated for symptoms using a simple system of absent (no water soaking or necrosis) or present (water soaking symptom around X, or pattern of necrosis around X).

### 3.7 Cantilever Brush Configuration Experiment

Four different pairs of cantilever brushes were provided by USDA-ARS-CPPRU engineers to investigate if delinting time could be improved by using different brushes. The brushes included different wire diameters in both wound and stacked configurations. The brush wire diameters were .0012 in, .0008 in, and .0017 in for the wound wire design. The stacked wire brush design had only the .0012 in wire diameter. With these cantilever brushes, a total of 16 combinations were possible. Each cantilever brush combination was assigned a number from 1-16 and randomized. Each brush combination was used to delint four 80 g samples, after which the delinter was cleaned. This was repeated four times to complete four replications. The time to delint each sample was recorded. Each configuration was run according to the brush randomization.

The cantilever brushes were changed by first removing the seed collection chamber (Figure 2.C), front face plate (Figure 2.E), and the drum (Figure 3). Next, all the belts were removed from the pulleys (Figure 8.A),

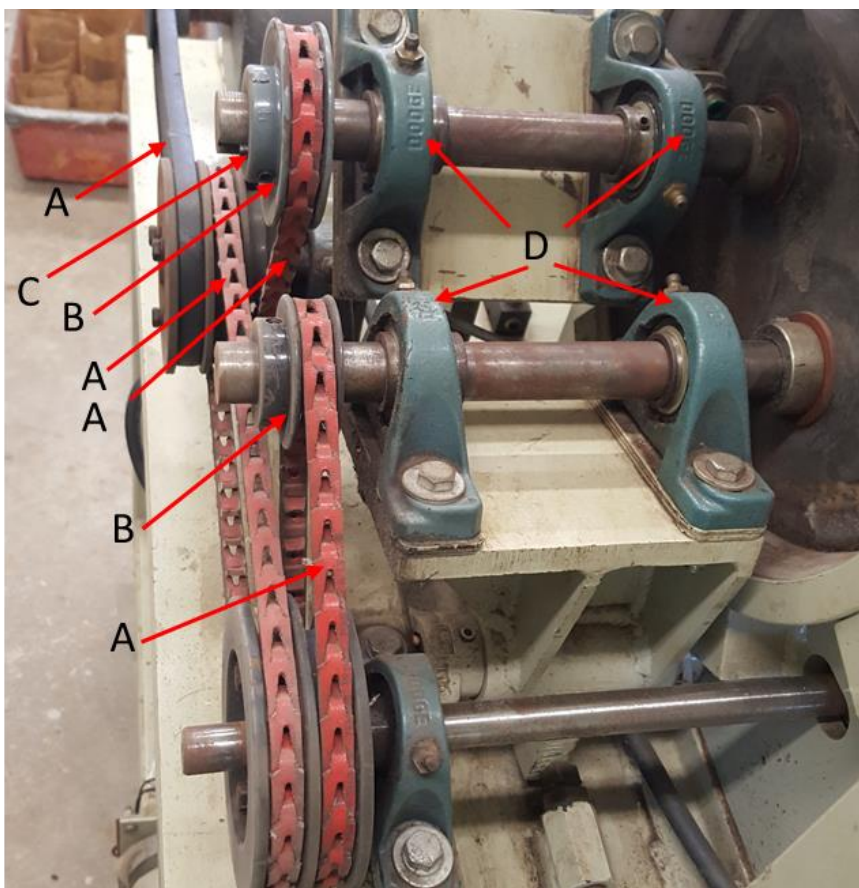


Figure 8. Image of mechanical cottonseed delinter power transmission system with pulley and belt configuration with individual components. A.) Belts; B). Pulleys; C.) Square key stock; D.) Bearings.

and the top two pulleys (Figure 8.B) were removed by loosening the set screws using hexagonal Allen wrenches and removing the pulleys and the key square stock (Figure 8.C). Once the pulleys were removed, the remaining set screws located on the bearing (Figure 9.A) were loosened using hexagonal Allen wrenches.

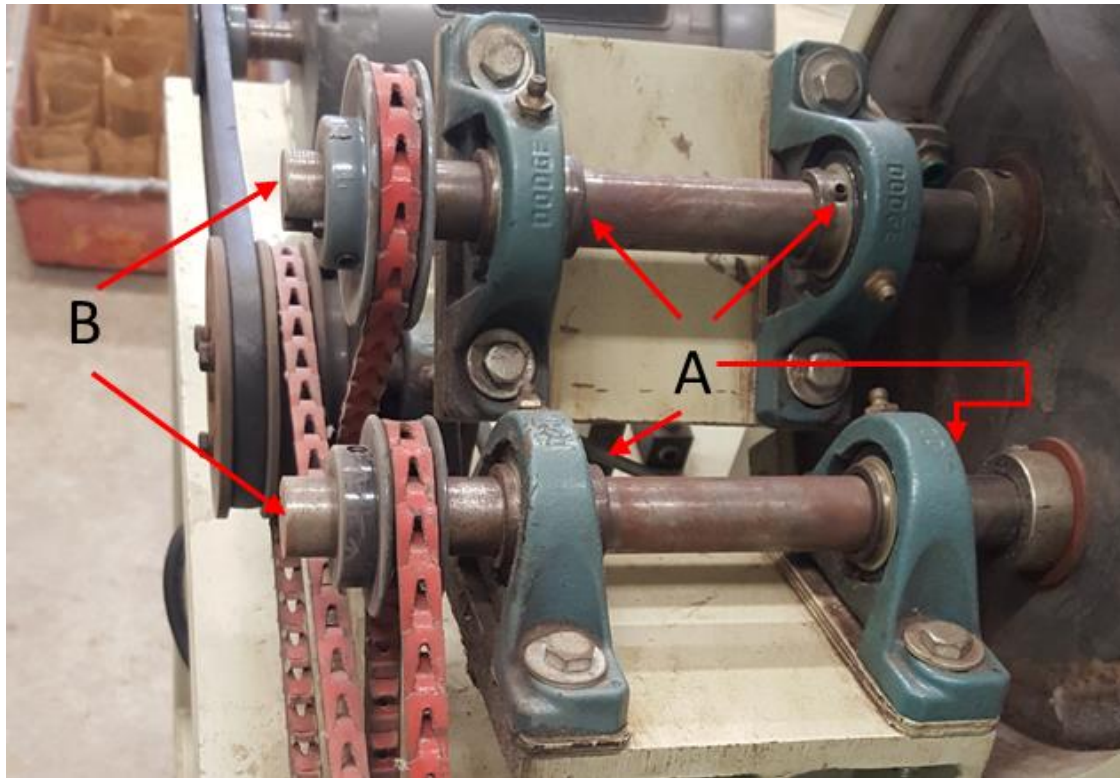


Figure 9. Image of mechanical cottonseed delinter power transmission system and components requiring removal to change brushes. A.) Set screws on Bearings; B.) End of shafts.

After all the set screws located by the bearing had been loosened, the last set screws located on the collars also were loosened to the point that the collars would move freely (Figure 10.A).

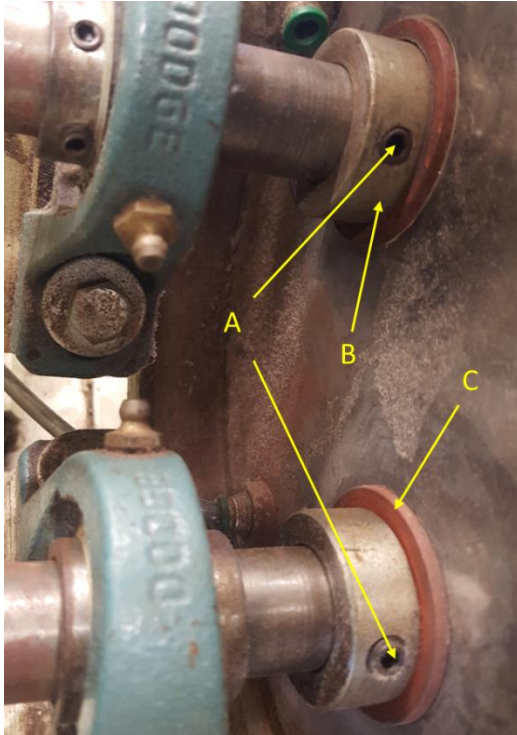


Figure 10. Image of mechanical cottonseed delinter set screw and collar locations. B.) Collars; C.) Fire block washers.

At this point, the brushes were removed by striking the end of the shaft with a dead blow rubber mallet. Once the brushes were moved far enough for fingers to be placed between the back of the brushes and the back-face plate, the brushes manually were pulled off. After the brushes were removed, the collars (Figure 10.B) and fire block washers (Figure 10.C) were collected. The next combination of brushes was then put in place by first inserting the brushes into the holes in the back-face-plate (Figure 4.B). Before the brush was inserted into the bearing, red fire block washers (Figure 10.C) and collars (Figure 10.B) were placed on the shaft. After the washer and collar were placed on the shaft, the shaft was then inserted into the bearing (Figure 8.D). The brushes were placed with enough space between the back of the back-face plate (Figure 4.B) and the back of the brush so as not to impede the turning of the brushes. When the brushes were positioned in the desired location, the set screws were tightened, and the red

fire block washer and collar were set against the outside of the back-faceplate and tightened. After the set screws on the shaft were set, the pulleys were placed on the shaft by aligning the key and the key slot. The belts were put back and once the belts were replaced, the pulleys were adjusted to align the belts to ensure proper movement and the set screws on the pulleys were tightened.

After the brush combination had been changed, the delinter was reassembled and that combination was tested. After the delinting times from the experiment were recorded, data from the experiment was analyzed using a standard least squares model and a means comparison in JMP 12 Pro. Tables were made by exporting reporting from JMP Pro 12 into Microsoft Excel.

### 3.8 Variable Seed Size Experiment

In 2016, 16 genotypes with different linter densities and seed sizes were selected and planted in two-row, 8 m long, plots using a Case International 900 plot planter at the LREC. A random 25-boll sample was taken from each genotype and ginned on tabletop gins. After the genotypes were ginned, a seed index and a linter density were recorded for each genotype. Eight samples were selected based upon variation in the recorded seed indexes (SI) and linter densities and saved for a seed increase in 2017. Linter density did not vary appreciably so selections were made based on seed index. The seven genotypes used for testing are in Table 2.

Table 2. Genotypes selected for the mechanical cottonseed delinter variable seed size experiment with their corresponding entry number and seed index.

Entry	Genotype	Seed Index
1	15-1-506	8.7
2	15-3-416	13.8
3	15-3-920	10.8
4	15-1-109	8.5
5	15-3-724	12.6
6	FM 2011GT (PVP201100382)	8.1
7	DP 1219 B2RF (PVP201100260)	7.6

The eighth entry was not included in the testing due to excessive mechanical damage during ginning, and high segregation. Entries 1-5 are unreleased breeding lines from LREC cotton breeding program selected for strains testing in 2015. In 2017, the seven selections for seed increase were planted in two-row plots, 8 m long with four replications using a Case International 900 plot planter at the LREC. The machine harvested increase samples were then ginned using the laboratory research gin at the LREC.

The ginned seed samples from each genotype were randomly delinted. Each sample was delinted and the time to achieve visually smooth seed was recorded. For each genotype, four 100 g samples were processed, then the delinter was cleaned between genotypes. This process was replicated 4 times. A null hypothesis was developed to investigate whether delinting time differs depending on seed size.

The null hypothesis stated that delinting time would not differ based on seed size. After the data from the experiment were recorded, it was analyzed using a standard least squares model and a means separation in JMP 12 Pro. The correlation figure was also generated using JMP Pro 12. Tables were generated by exporting the JMP Pro 12 analysis into Microsoft Excel, and Figure 17 was made using Microsoft Excel.

### 3.9 Timed Weight Experiment

The weight experiment consisted of five sample sizes, five mechanical delinting times and an acid delinting control. Samples sizes included a counted sample of 100 seed (seed index 9.2), and four weighed samples of 100 g, 200 g, 300 g, and 400 g. The sample delinting times were 3 min, 4 min, 5 min, 6 min, and 7 min. The experiment was deployed as an RCBD with 3 replications with two factors being delinting time and sample size. The samples were

mechanically delinted corresponding to the assigned size and time combinations. The delinter drum was cleaned after every five samples. The control weighed samples were acid delinted until completely free of linters, but time was not considered since the efficacy of acid delinting can change and deteriorate over time. The weight loss due to delinting was compared between mechanically delinted seed and the control samples (acid delinted).

This experiment was adjusted and repeated using a new brush combination (combination 16) from the cantilever brush combination experiment. The weight loss due to delinting was compared between mechanically delinted seed and the control samples (acid delinted). The same control samples were used for this comparison. The experimental design and null hypothesis were the same as the original timed weight experiment. Data sets from both experiments were analyzed using JMP 12 Pro as a two-way factorial RCBD. Tables were generated by exporting the JMP 12 Pro reports into Microsoft Excel. Figures also were generated using Microsoft Excel.

## 4. RESULTS

### 4.1 Optimum Timing Experiment

The first execution of the optimum timing experiment rejected the null hypothesis of no difference in delinting time between samples of different weights based on a highly significant p-value of 0.0001 and indicated delinting time differed based on the size of the samples (Table 3). Regression analysis determined a relationship between sample size and delinting time. The linear regression model had a R-squared value of 0.5483 and the slope of the regression line was 0.2623. The positive slope of the regression line indicates that delinting time does increase with sample size. The means for delinting time ranged from 607.5 seconds to 471.5 seconds. Means comparison analysis shows a Least Significant Difference of 26.9 seconds. Mean separations show four clean levels of separation in the time required to delint the different sample sizes (Table 4). Results of the germination test revealed that the average germination for the seed from this experiment was 86 %. The correlation between sample size and time was calculated to be 0.74 with the model explaining approximately 75 percent of the variation.

Table 3. Analysis of variance for delinting time of different sample sizes (50-g, 100-g, 150-g, 200-g, 250-g, 300-g, 350-g, 400-g, and 450-g) of the mechanical cottonseed delinter optimum timing experiment in 2016.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Sample	8	61975.056	7746.88	22.804	<.0001
Rep	3	5178.083	1726.03	5.0808	0.0073
Error	24	8153.167	339.72		
C. Total	35	75306.306			

\* significant, \*\* highly significant



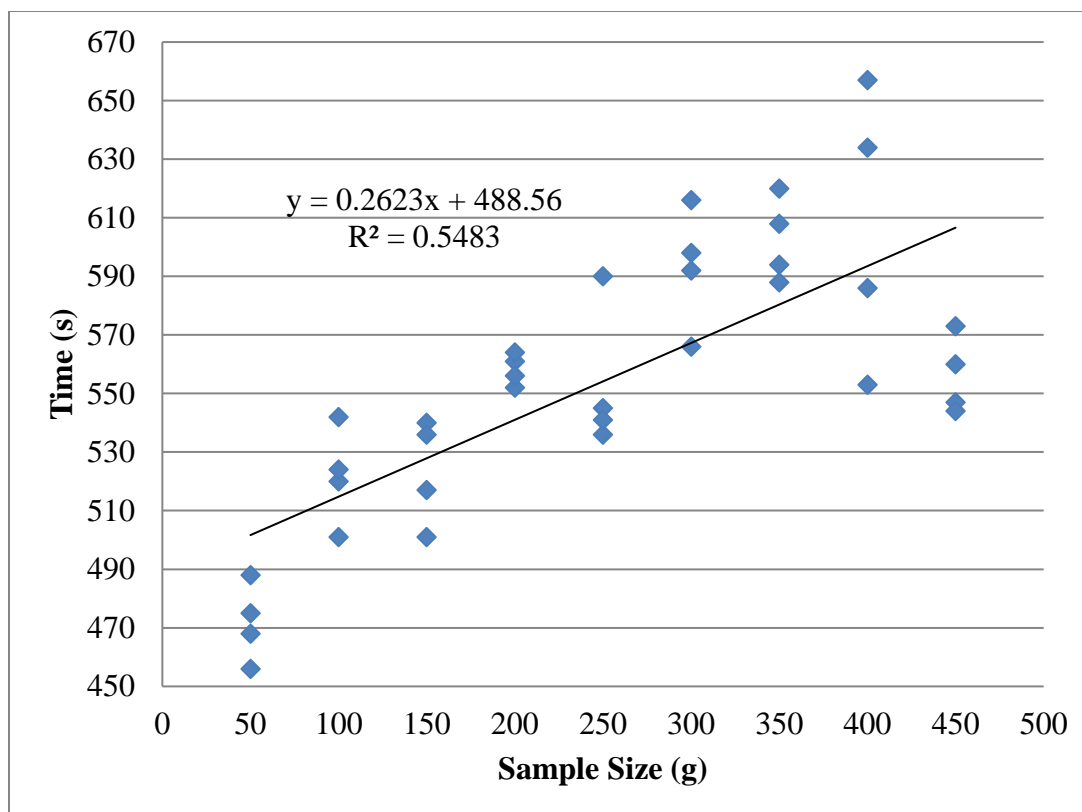


Figure 11. Scatterplot of samples sizes (g) and corresponding delinting time (s) for the 2016 mechanical cottonseed delinter optimum timing experiment.

Table 4. Least squares means for times (s) of various samples sizes (g) for the 2016 mechanical cottonseed delinter optimum timing experiment.

Sample Size (g)		Mean (s)
400	A <sup>†</sup>	607.50
350	A	602.50
300	A	593.00
200	B	558.25
450	B	556.00
250	B	553.00
150	C	523.50
100	C	521.75
50	D	471.75

<sup>†</sup> Means with same letters are not significantly different

In the second duplication of the experiment, results of the ANOVA suggested we should reject the null hypothesis based on a highly significant p-value of 0.0001 and indicated delinting time differs based on the size of the samples (Table 5). A linear regression model had a R-squared value of 0.6998 and the slope of the regression line was 0.2368. The means for delinting time ranged from 396.5 seconds to 284.3 seconds. Means comparison analysis gives us a Least Significant Difference of 13.8 seconds. Mean separation shows four levels of separation in the time required to delint for the different sample sizes (Table 6). The correlation coefficient for this trial was calculated to be 0.83 making sample size and delinting time moderately correlated.

Table 5. Analysis of variance for delinting time of different sample sizes (50-g, 100-g,-150-g, 200-g, 250-g, 300-g, 350-g, 400-g, and 450-g) of the mechanical cottonseed delinter optimum timing experiment in 2018.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Sample	8	45260	5657.5	63.3434	**
Rep	3	685.444	228.5	2.5582	0.0788
Error	24	2144	89.3		
C. Total	35	48089			

\* significant, \*\* highly significant

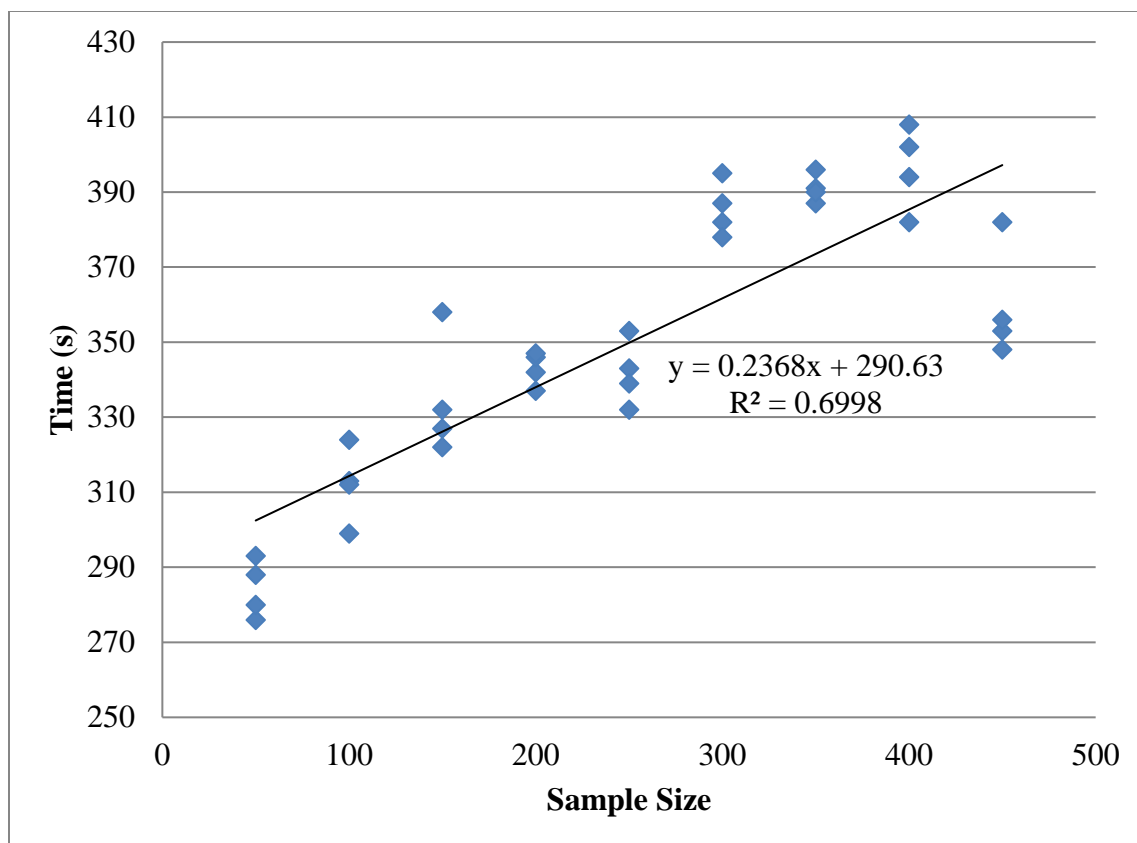


Figure 12. Scatterplot of samples sizes (g) and corresponding delinting time (s) for the 2018 mechanical cottonseed delinter optimum timing experiment.

Table 6. Least squares means for times (s) of various samples sizes (g) for the 2016 mechanical cottonseed delinter optimum timing experiment.

Sample Size (g)		Mean (s)
400	A <sup>†</sup>	396.50
350	A	391.00
300	A	385.50
450	B	359.75
200	C	343.00
250	C	341.75
150	C	334.75
100	D	312.00
50	E	284.75

<sup>†</sup> Means with same letters are not significantly different

There are a complexity of factors that could influence delinting time, so it would be a poor idea to try to predict based on the sample size alone. The regressions tell us the time to delint will increase as the sample size increases. The trend is true except for the largest sample size of 450g, which fell in the middle of the rankings in the means separation (Table 6). This phenomenon may likely be due to the linters not being removed and creating a cylindrical mass of linter possibly creating more friction and thus decreasing delinting time.

#### 4.2 Seed Carry-Over Experiment

A series of experiments were conducted to determine if the amount of seed carry-over could be reduced using different drum treatments. The p-value from the 2016 experiment using only the stock drum was calculated to be 0.0083 (Table 7). This identifies that the amount of seed carry-over was significantly different than 0, which is the threshold—above which is unacceptable in breeding programs. Figure 13 shows averages of first, second, third and fourth samples delinted to show any time relationships as the experiment progressed. Figure 13 shows a maximum seed carry-over of 1.27 percent for the averaged first samples delinted. The amount of carry-over after the first sample decreased to 0.16 percent and continues to decrease for every sample after that. Although the seed carry over numbers seem low, cumulative seed carry-over of 1.27 percent from every sample delinted is unacceptable. The average germination of the delinted seed with the stock drum was 90.19%.

Table 7. P-values for seed carry-over counts for each mechanical cottonseed delinter drum treatment (normal, packed, finished and finished drum with stacked brushes) in 2016, 2017 and 2018.

Year	Treatment	P-value	Significance
2016	Normal	0.01	Significantly different than 0
2017	Packed	0.03	Significantly different than 0
2017	Finished	0.06	Not significantly different than 0
2018	Finished drum with Stacked Brushes	0.03	Significantly different than 0

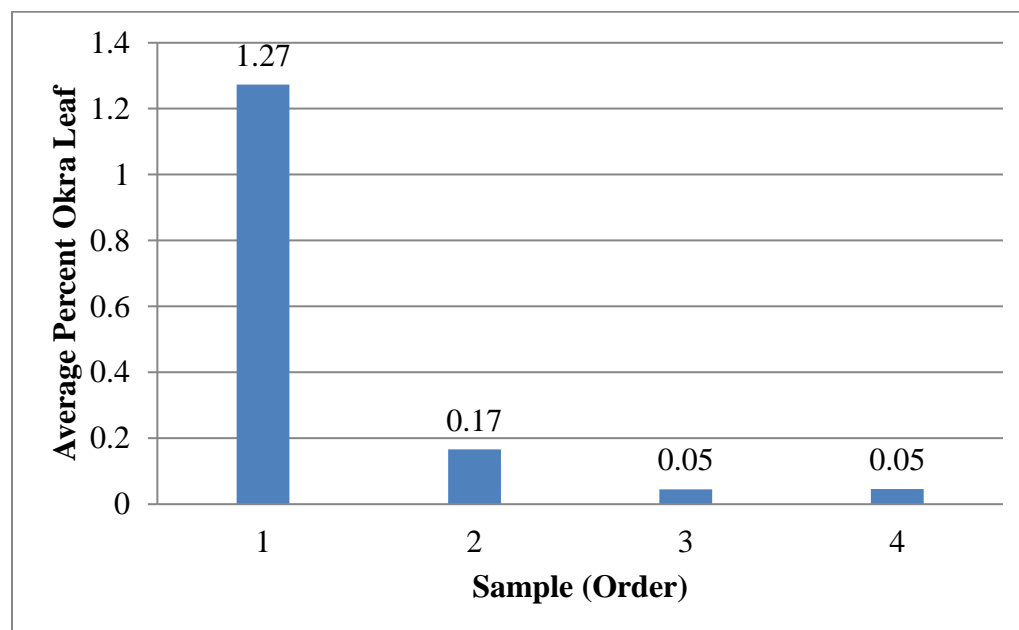


Figure 13. Average seed carry-over for the first, second, third and fourth samples delinted with mechanical cottonseed delinter using a normal drum. In 2016, samples were planted, and counts taken in a greenhouse.

In 2017, the experiment comparing the packed drum and the finished drum treatment was conducted. The experiment differed from the previous year in that delinted seed were planted in a field environment. The experiment shows that maximum seed carry-over of 0.58 percent in the

packed drum (Figure 15). The amount of carry-over after the first sample decreased to 0.31 percent and continued to decrease for subsequent samples. The p-value for the packed drum treatment was 0.03, significantly different from 0 and over the acceptable threshold (Table 7). The experiment with the finished drum treatment shows a maximum seed carry-over of 0.32 percent (Figure 14). The amount of carry-over with the finished drum treatment after the first sample stayed constant with the minimum seed carry over 0.16 percent. The field germination for the packed drum treatment was calculated to be 84.54 percent. The finished drum p-value of 0.06 shows that there was seed carry-over, but the amount of seed carry-over was not significantly different than 0 at 95 percent certainty (Table 7). The field germination for the finished drum treatment was calculated to be 76.19%. The p-value may suggest the finished drum treatment may not carry seed from sample to sample, but this is most likely not the case. Examination of the raw numbers reveal that an okra leaf occurrence was present. Though not statistically significant, that occurrence is beyond the acceptable threshold of zero. This experiment shows carry-over from one sample for up to four more samples following it before cleaning. When processing breeding samples, any carryover from the immediately preceding sample is unacceptable cross-contamination.

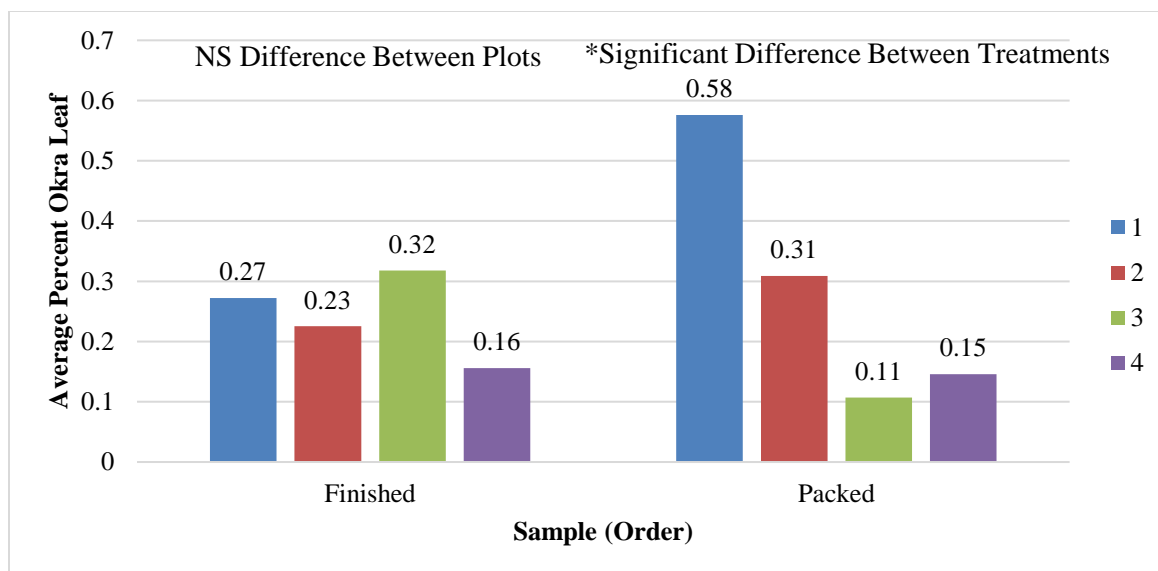


Figure 14. Average seed carry-over for the first, second, third and fourth samples delinted with mechanical cottonseed delinter using a finished and a packed drum. In 2017, samples were planted, and counts were taken in a field located at LREC.

In 2018, the experiment was conducted using the finished drum with the stacked brushes. Carry-over was evaluated by planting the delinted samples in a field environment. The experiment showed a maximum seed carry-over of 1.47 percent. The amount of carry-over after the first sample decreases to 0.42 percent and then averaged around 0.7 percent for the last two means. The p-value for the packed drum treatment was 0.03 significantly different from 0 (Table 7). The drum treatment and brush treatment appear to have increased the amount of seed carry over from the previous year's finished drum treatment. The 2018 field germination was very poor with the germination of samples ranging from 43.71 percent to 62.77 percent with an average of 50.50 percent. In 2018, an additional drum treatment was delinted and planted but due to some planting errors, the data were not collected.

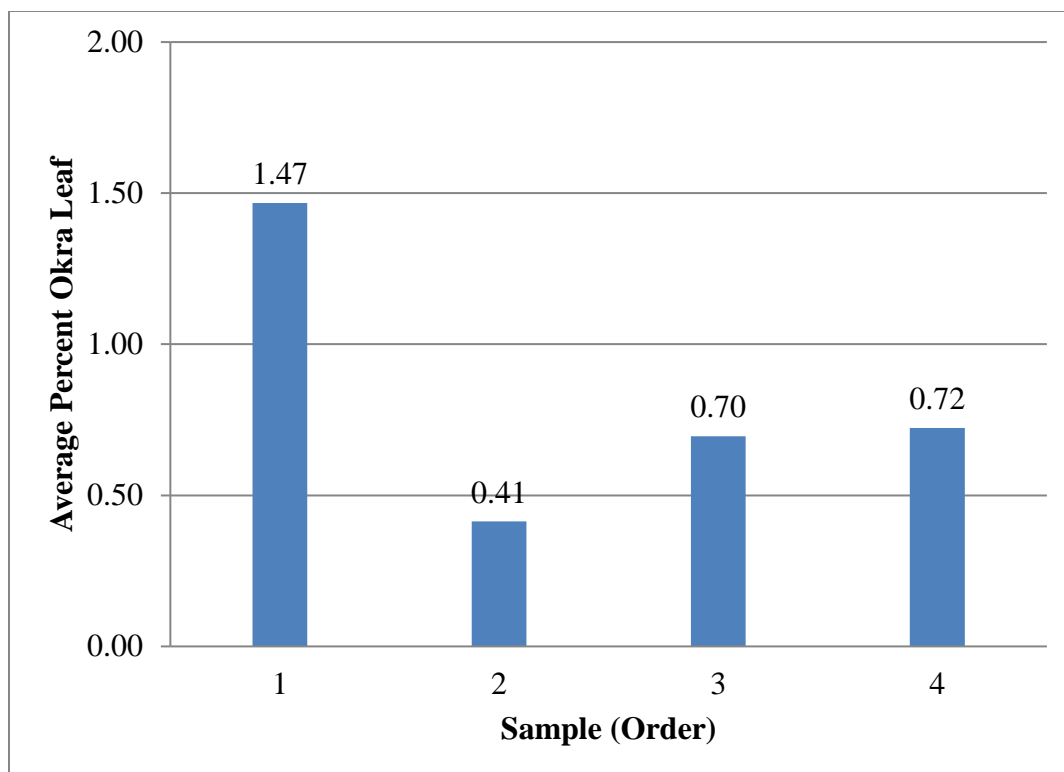


Figure 15. Average seed carry-over for the first, second, third and fourth samples delinted with mechanical cottonseed delinter using a finished drum with a stacked cantilever brush design. In 2018, samples were planted, and counts were taken in a field located at LREC.

#### 4.3 Seed-Borne Disease Experiment

Acid delinting of cottonseed is effective in preventing seed transmission of bacterial blight (Smith and Cothren, 1999; and Drummond and Savoy, 1996), which may not occur with mechanically delinted seed. From the plants that were inoculated in 2016, mean number of symptomatic plants for the mechanically delinted samples was 8.6 and the acid delinted samples had a mean of 6.5. The p-value of 0.62 (Table 8) shows that the two delinting treatments are not significantly different.



Table 8. F-test results for the number of *Xanthamonas* symptomatic plants originating from acid delinted versus mechanically delinted cottonseed samples in 2016.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Method	1	6.30	6.30	0.28	0.62
Error	5	113.70	22.74		
C. Total	6	120.00			

\* significant, \*\* highly significant

From the plants that were inoculated in 2017, the mean number of symptomatic plants for the mechanically delinted samples was calculated to be 2.13 and the acid delinted samples had a mean of 1.14. The p-value of 0.26 (Table 9) shows that the two delinting treatments are not significantly different.

Table 9. F-test results for average *Xanthamonas* symptomatic plants originating from acid delinted versus mechanically delinted cottonseed samples in 2017.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	4.68	4.68	1.33	0.26
Error	20	70.59	3.53		
C. Total	21	75.27			

\* significant, \*\* highly significant

From the seed that was plated on the potato dextrose agar, the mean number of colonies for the mechanically delinted samples was calculated to be 59.43 and the acid delinted samples had a mean of 5.14. The p-value of 0.0091 (Table 10) shows that the two delinting treatments were significantly different, though due to the large number of occurrences, not all the counted bacteria colonies could be confirmed.

Table 10. F Table for *Xanthamonas* colonies on petri dishes for an acid delinted cottonseed sample and a mechanically delinted cottonseed sample.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
treatment	1	10314.29	10314.3	9.63	*
Error	12	12852.57	1071		
C. Total	13	23166.86			

\* significant, \*\* highly significant

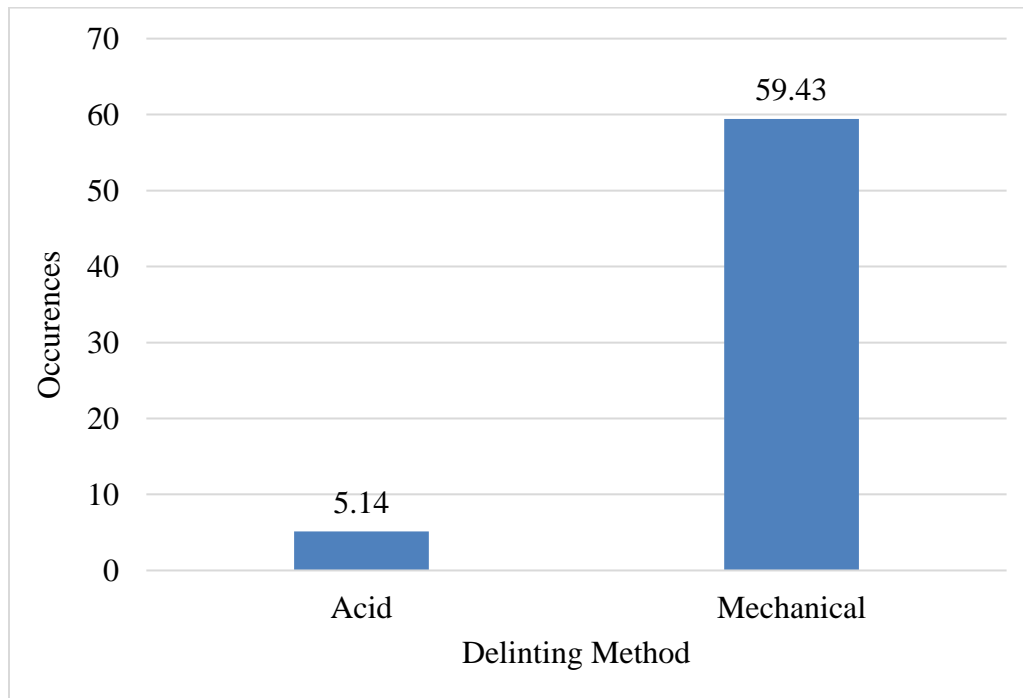


Figure 16. Mean of counted *Xanthamonas* colonies on petri dishes for an acid delinted cottonseed sample and a mechanically delinted cottonseed sample.

#### 4.4 Cantilever Brush Configuration Experiment

Sixteen different brush cantilever brush combinations were examined to determine if delinting time could be reduced. The null hypothesis stating that delinting time would not differ between different brush designs was rejected based on a highly significant p-value of 0.0001 shown in Table 11 indicating that delinting time differs based on the configuration of the brushes.

The means for delinting time ranged from 353 seconds to 164 seconds. Mean comparison analysis gave a Least Significant Difference of 8.28 seconds and found the time to delint all the genotypes are significantly different regardless of seed size. The original brush configuration Combination 1 comprised of 2, 0.0012-in wound wire had a mean delinting time of 284.5 seconds. The delinting time for the best performing and stand-alone significantly higher brush combination (combination 16) was the stacked brushes with a wire diameter of 0.0012 in. was 164.5 seconds (Table 12). Combination 16 reduced the time to delint by 57 percent compared to the original brush which was configuration 1. With this information, combination 16 is recommended in further designs for mechanically delinting small breeder samples.

Table 11. Analysis of variance of delinting time (s) for 16 different cantilever brush combinations on mechanical cottonseed delinter.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Combination	15	778668.78	51911.30	367.38	**
Rep	3	915.70	305.20	2.16	0.09
Error	237	33487.99	141.30		
C. Total	255	813072.46			

\* significant, \*\* highly significant

Table 12. Comparison of mean delinting time for individual samples for 16 brush combinations on mechanical cottonseed delinter.

Combination	Left Brush	Right Brush		Mean
3	Original Brush	0.008 Wound Brush	A <sup>†</sup>	353.938
2	Original Brush	0.017 Wound Brush	B	335.875
6	.017 Wound Brush	0.017 Wound Brush	B	334.250
1	Original Brush	Original Brush	C	284.500
5	.017 Wound Brush	Original Brush	C D	277.250
4	Original Brush	Stacked Brush	D	275.000
7	.017 Wound Brush	0.008 Wound Brush	E	261.563
10	.008 Wound Brush	0.017 Wound Brush	E	257.063
9	.008 Wound Brush	Original Brush	F	242.875
8	.017 Wound Brush	Stacked Brush	F	239.813
15	Stacked Brush	0.008 Wound Brush	G	218.938
11	.008 Wound Brush	0.008 Wound Brush	G	218.563
12	.008 Wound Brush	Stacked Brush	G	212.375
14	Stacked Brush	0.017 Wound Brush	H	180.563
13	Stacked Brush	Original Brush	H	178.688
16	Stacked Brush	Stacked Brush	I	164.563

<sup>†</sup> Means with same letters are not significantly different

#### 4.5 Variable Seed Size Experiment

After seed increases from genotypes selected for variation in seed size and linter density were ginned, the linter densities among them did not vary so the effects of linter density were not examined. Based on the ANOVA, the null hypothesis of no differences was rejected indicating that delinting time differs among genotypes with different seed sizes (Table 13). The means for delinting time ranged from 332 seconds to 150 seconds. Means comparison analysis gave a Least Significant Difference of 5 seconds, with the time to delint for all the genotypes being significantly different from each other (Table 14). The correlation coefficient between seed size and delinting time is 0.2389 and the slope is near zero, so there is no trend or correlation (Figure 17, Figure 18). Factors other than seed index appear to be impacting delinting time among genotypes.

Table 13. Analysis of variance of mean delinting time to delint seven genotypes with varying seed sizes on a mechanical cottonseed delinter.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Entry	6	312869.75	52145	1006.57	**
Rep	3	64.74	22	0.42	0.7415
Error	102	5284.07	52		
C. Total	111	318218.56			

\* significant, \*\* highly significant

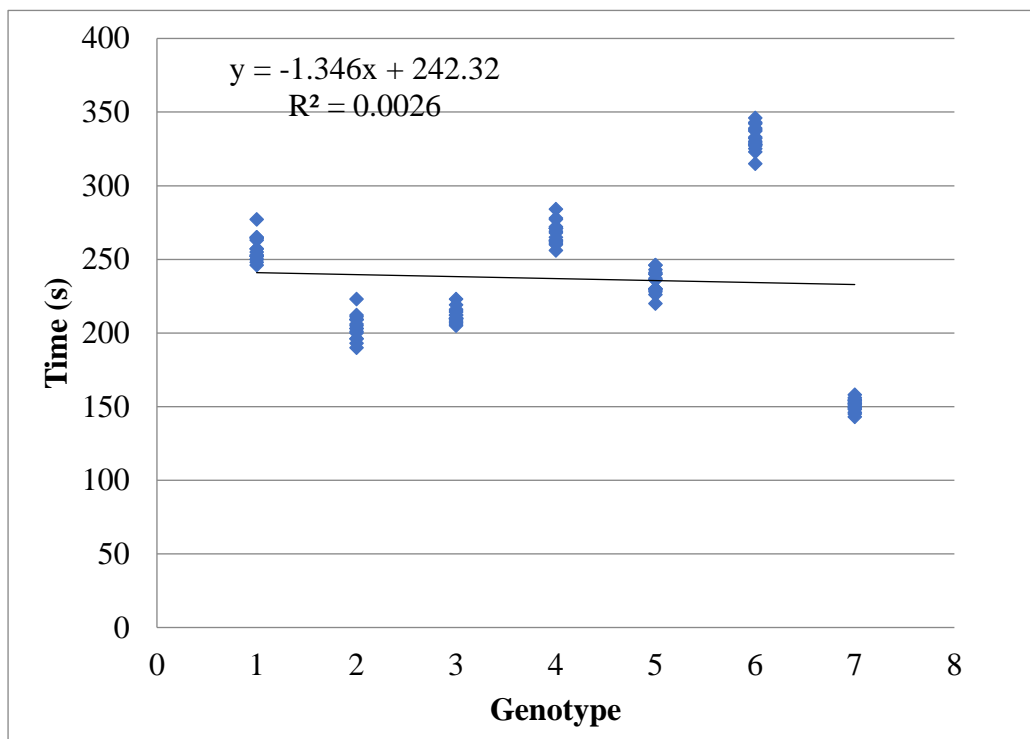


Figure 17. Scatterplot of genotypes and their corresponding time to delint (s) for the mechanical cottonseed delinter variable seed size experiment.

Table 14. Mean comparison of time (s) to delint seven genotypes with varying seed size on a mechanical cottonseed delinter.

Entry	Genotype	Seed Index	Mean
6	FM 2011GT	8.1	A <sup>†</sup> 332.188
4	15-1-109	8.5	B 268.188
1	15-1-506	8.7	C 256.625
5	15-3-724	12.6	D 234.875
3	15-3-920	10.8	E 211.625
2	15-3-416	13.8	F 204.125
7	DP 1219B2RF	7.6	G 150.938

<sup>†</sup> Means with same letters are not significantly different

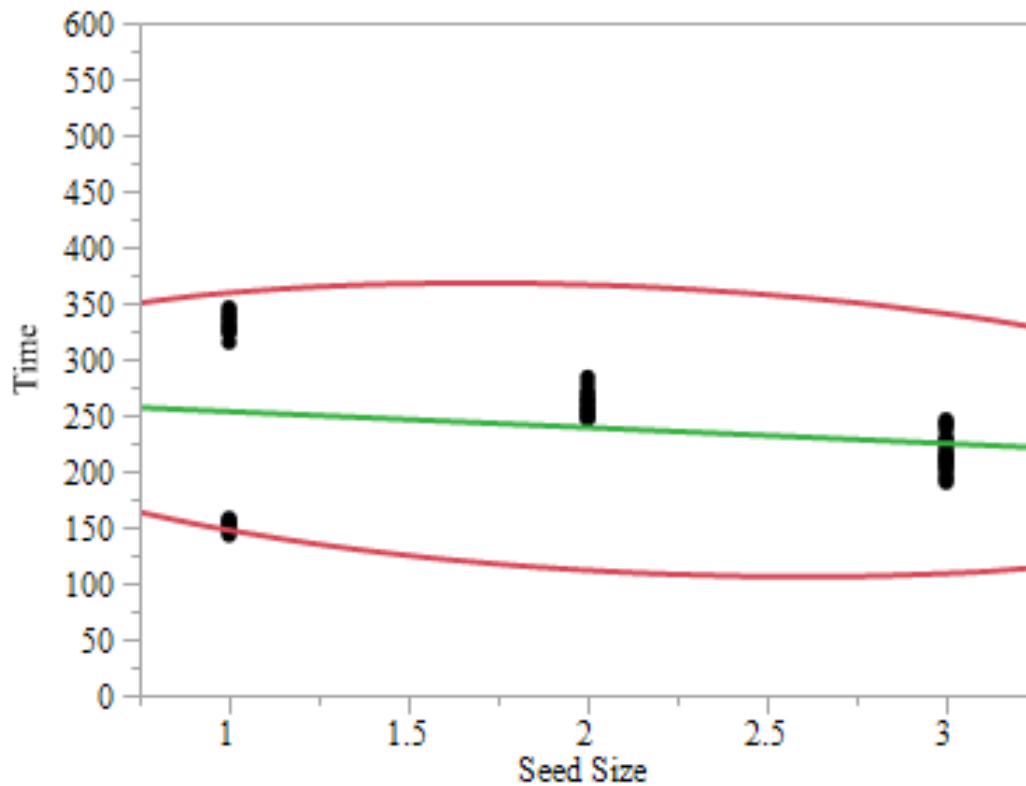


Figure 18. Correlation between three seed sizes (1 is small, 2 is medium, and 3 is large) and time to delint (s) a 100 g sample on a mechanical cottonseed delinter.

#### 4.6 Timed Weight Experiment

The timed weight experiment included two factors; a set delinting time and a set sample size (weight). Each time-weight combination was delinted and the percent weight loss after delinting was calculated. For the timed experiment, across all sample sizes the average weight loss per sample was 12 %. The ANOVA from the first experiment recommended rejection of the null hypothesis based on a highly significant p-value of 0.0049 and indicated that delinting time differs based on the size of the samples (Table 15). The means for percent weight loss when considering the delinting time ranged from 11.66 percent to 16.96 percent.

Table 15. Analysis of variance of average weight loss during delinting for five delinting times and five sample sizes for the 2016 mechanical cottonseed delinter timed weight experiment.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	24	884.06	36.84	2.38	*
Error	50	774.21	15.48		
C. Total	74	1658.28			

\* significant, \*\* highly significant

Sample size and the interaction between the sample size and set delinting time were both nonsignificant (Table 16). The set delinting time was significant with a p-value of 0.0018.

Table 16. Effect of sample size, delinting time and sample size \* delinting time on average weight loss from mechanical cottonseed delinter in 2016.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Sample	4	4	124.91	2.02	0.11
Time	4	4	310.69	5.02	*
Sample*Time	16	16	448.45	1.81	0.06

\* significant, \*\* highly significant

The P-value from the ANOVA testing delinting time and size of samples using the stacked brush design recommends rejection of the null hypothesis based on a highly significant p-value of 0.0049 and indicates that delinting time differs based on the size of the samples (Table 17). These results agree with results from the first performance of this same experiment. The means for percent weight loss range from 12.07 percent to 17.39 percent.

Table 17. Analysis of variance of average weight loss during delinting for five delinting times and five sample sizes for the mechanical cottonseed delinter timed weight experiment with stacked cantilever brushes.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	24	3692.51	153.86	13.61	**
Error	50	565.11	11.30		
C. Total	74	4257.62			

\* significant, \*\* highly significant

The effects tests indicate that the interaction between the sample size and set delinting time was not significant (Table 18). The set delinting time was significant with a p-value of 0.0018 and the sample size was significant with a p-value less than 0.0001.

Table 18. Effect of sample size, delinting time and sample size \* delinting time on average weight loss from mechanical cottonseed delinter with stacked cantilever brushes in 2018.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Sample	4	4	3258.84	72.08	**
Time	4	4	234.05	5.18	*
Sample*Time	16	16	199.61	1.10	0.38

\* significant, \*\* highly significant

The original experiment tested if weight loss would not differ between samples of different weight and time. The ANOVA indicates that we can reject the null hypothesis based on



a highly significant p-value of 0.0049 and indicates that delinting time differs based on the size of the samples (Table 19). The means for percent weight loss ranged from 11.91 percent to 14.8 percent.

Table 19. Analysis of variance of average weight loss during delinting for five delinting times and five sample sizes for the mechanical cottonseed delinter timed weight experiment, 2016 and timed weight experiment with stacked cantilever brushes, 2018.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	49	4592.66	93.73	7.00	**
Error	100	1339.33	13.39		
C. Total	149	5931.98			

\* significant, \*\* highly significant

The effects test show that the delinter brush configuration is not significant. The non-significant brush configuration suggests that different brushes do not affect the percent weight loss per sample size and a set delinting time (Table 20). The interaction between the sample size and set delinting time were both not significant. The set delinting time was significant with a p-value of 0.0018.

Table 20. Effect of configuration, sample size, delinting time, configuration \* sample size, configuration \* delinting time, sample size \* delinting time, and configuration \* sample size \* delinting time on average weight loss from mechanical cottonseed delinter.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Configuration	1	1	16.08	1.20	0.28
Sample	4	4	1094.87	20.44	**
Time	4	4	274.64	5.13	*
Configuration*Sample	4	4	2288.89	42.72	**
Configuration*Time	4	4	270.11	5.04	*
Sample*Time	16	16	272.82	1.27	0.23
Configuration*Sample*Time	16	16	375.25	1.75	*

\* significant, \*\* highly significant

## 5. DISCUSSION

### 5.1 Optimum Timing Experiment

It was determined that as sample size increases, the time to mechanically delint cottonseed increases, with the exception of the 450 g sample which was the largest sample tested. The relationship between the delinting time and weight was expected. The correlation was expected because of the increased amount of seed that needs to be delinted. The decrease of delinting time for the 450 g samples was not expected and can be explained. While delinting the 450 g samples, a cylindrical mass of lint was generated that was approximately 2.5 cm in diameter and stretched the length of the drum. This cylindrical mass may have attributed to the decrease in delinting time. Further research is needed to determine if larger, seed increase samples can be more efficiently delinted mechanically compared to smaller boll sample or individual plant selection which would fall between 20 g and 200 g.

### 5.2 Seed Carry-Over Experiment

Evaluating the possibility of modifying the mechanical delinter design to decrease the seed carry-over between samples is one of the most important components of determining the feasibility of this processing method for breeding programs. Due to the many different genotypes handled and processed by cotton breeding programs, the elimination of excessive cleaning to prevent seed carry-over would greatly improve the efficiency of operations. Even though the percentage of carry-over is low, ideally any carry-over is unacceptable. This ideal may not be attainable, so a minimum may be all that can be achieved. In the current investigation, none of the various drum treatments reduced the carry-over enough to be considered as a solution. The finished drum treatment, as well as the packed drum treatment, indicated the voids between brushes, which is the suspected source of carry-over, may not be the

only source due to the voids being filled. Other sources of seed carry-over also may be involved, but further investigation will be needed to determine the remaining sources. Results indicate that additional improvements can be made to reduce seed carry-over. The current mechanical delinter design could only be used in projects where some seed mixture can be tolerated, or clean-out can be performed between samples.

### 5.3 Seed-Borne Disease Experiment

The transfer of seed borne-disease on the surface of the seed was not seen to differ based on the delinting method. While there were no differences between the two delinting methods, it cannot be said that mechanically delinting seed removes seed-borne diseases better than acid delinting. Acid delinting is the most commonly use method to remove linters and also partially removes potential pathogens during the process. For mechanical delinting to be used in a cotton breeding program, a system to reduce pathogens from the surface of the seed would need to be implemented. There are other ways to control *Xanthamonas* such as antibacterial treatments, but research would have to be conducted to ensure that there are no adverse effects to treating cottonseed with such an approach. Otherwise, the mechanical delinter should only be used for samples known to come from disease-free nurseries.

### 5.4 Cantilever Brush Configuration Experiment

The original brush configuration equipped on the delinter fully delints cottonseed. The time required to reach a full polish is considerable compared to acid delinting. For the mechanical delinter to be able to handle the amount of volume that would be required of it, the delinting time would have to be reduced considerably, or more delinting heads added to the unit. The mechanical delinter was used by the Lubbock Cotton Improvement Program to process

cottonseed samples during the 2017 delinting season while other samples were being acid delinted. The difference in efficiency of each method was easily discernible.

The acid delinting required four people delinting while the mechanical delinter required one. The acid delinter processed approximately 60 samples while the mechanical delinter was able to delint 12 samples in the same time period. The samples that each method delinted were individual plant selections of varying sizes. The need for improved delinting time led to the design and construction of different brushes in hopes of reducing the delinting time. The stacked design shows the lowest delinting time with an average delinting time of 164 seconds.

The delinting time is the product of friction; because of this, the delinting time could be improved further if the amount of friction can be increased. The amount of friction could be improved by increasing the surface contact area between the brushes and the seed by adding more bristles to the cantilever brushes. The brush pitch placement could improve the time by closing the gap between the cantilever brushes and brushes on the drum.

### 5.5 Variable Seed Size Experiment

The variable seed size experiment was conducted to see if the delinting time differed based on the size of the seed. The analysis illustrated that the time required to delint a sample varied not based on the seed index but on the genotype being delinted. There was no correlation between seed index and time required to delint. A factor that could be attributed to the time required to delint a sample is the linter attachment force. Research has shown that different cultivars have different lint attachment forces (Bechere, Zeng, and Hardin, 2016). A high lint attachment force would make the linters still attached to the seed harder to remove, requiring more time to delint. Another factor could be the linter density on the seed. The linter density may increase or decrease delinting time depending on the number of attached short fibers

meaning that seed with a higher linter density may take longer to delint and vice versa. The linter density hypothesis was not tested because when the selections were increased and ginned, the linter densities did not vary, and all the increased seed had a medium linter density.

#### 5.6 Timed Weight Experiment

The timed weight experiment illustrates the percent weight loss will vary based on the length of time the sample is delinted because, unsurprisingly, the longer a sample is delinted, more linters are removed. Also, the average percent weight loss is not significantly different across sample size. The first attempt at the experiment showed that the percent weight loss of the longest delinting time and the shortest delinting time did not differ. Visual inspection of the post delinted samples does not support this (Figures 19 and 20).



Figure 19. Delinted cottonseed from a 100 g sample delinted with a mechanical cottonseed delinter for 3 min from the 2016 timed weight experiment.



Figure 20. Delinted cottonseed from a 100 g sample delinted with a mechanical cottonseed delinter for 3 min from the 2018 timed weight experiment.

These results perhaps can be attributed to user error, or non-delinted seed being removed from the drum by the air being improperly placed. These were the first experiments with the mechanical delinter, and the time spent executing the experiment resulted in trial-and-error experiences that improved familiarity with the machine. The knowledge gained included the volume of seed that can be delinted before cleaning was needed, how to adjust the air for maximum linter removal, and many other general maintenance concerns. The second attempt at this experiment showed the percent weight loss based on the delinting time decreased when delinting time was reduced as was expected. When the cantilever brushes were replaced, visual inspection of the seed showed the new brushes removed more of the linters than did the brushes that were worn.

## 5.7 Germination

Germination tests conducted with mechanical delinted cottonseed using the 4-day warm germination method found that the average germination was 94.2 percent with a range from 80 percent to 100 percent. The germination percent was calculated from the seed carry-over experiments. The seed from the carry-over experiment with the original drum was planted in cups in the greenhouse had an average germination of 90.19. In the experiments in 2018 the seed were planted in a field environment. The packed drum treatment had an average germination of 84.5 percent and the finished drum treatment had an average germination of 76.2. Acid delinted samples also were tested under similar conditions. Acid delinted seed were planted in cups in the greenhouse and the average germination of the seed was 92 percent. Acid delinted seed planted in the field was found to have an average germination of 88 percent. All the seed from the germination tests came from a common seed source. The average germination for the acid delinted samples were better than seed from the mechanically delinted samples, but the mechanical delinter is capable of producing samples with acceptable germination.

## 5.8 Cost of Acid Delinting Vs. Cost of Mechanical Brush Delinting

An issue for plant breeders is the cost of acid delinting. In 2018 acid delinting for the LREC cotton breeding program took approximately 200 hours. Based off the delinting facility located at the LREC, an estimated initial capital investment was calculated (Table 21).

Table 21. Initial capital investment for LREC cotton breeding seed delinting facility. Cost includes effluent holding and transferring equipment, personal protection equipment, chemical resistant delinting equipment and other miscellaneous items needed to acid delint cottonseed for a breeding program.

<b>Product</b>	<b>Quantity</b>	<b>Unit</b>	<b>Price</b>	<b>Extended Price</b>
Submersible Pump	1	Ea	\$190	\$190
Acid Resistant Mixer	2	Ea	\$360	\$720
Chemical Resistant Sink	1	Ea	\$4,934	\$4,934
Liquid Storage Tank (1550 Gallon)	2	Ea	\$800	\$1,600
Effluent Storage Trailer	1	Ea	\$12,250	\$12,250
Combination Emergency Eye Wash Station, and Shower	1	Ea	\$818	\$818
Tyvex Coveralls	10	Ea	\$12.67	\$126.70
Chemical Resistant Gloves	10	Ea	\$9.29	\$92.90
Face Shields	2	Ea	\$27.30	\$54.60
Shoe Covers	1	per 100	\$23.56	\$23.56
Custom Dryer Box	1	Ea	\$1,500.00	\$1,500.00
Miscellaneous Items	n/a	n/a	\$500	\$500
<b>Total</b>				<b>\$22,810</b>

Included in the initial investment are some recurring costs. This includes the submersible pump, Tyvex coveralls, face shields, chemical resistant gloves, and shoe covers. Many miscellaneous items were also included in the cost. The miscellaneous items include seed drying trays, seed delinting jars, seed delinting tubs for large samples, and various plumbing fittings.



Table 22. Recurring cost calculated from acid delinting cottonseed for breeding program in 2018 which includes labor and the amount of chemicals needed.

<b>Cost</b>	<b>Quantity</b>	<b>Unit</b>	<b>Price</b>	<b>Extended Price</b>
Labor for 4 Workers	200	hr	\$35.00	\$7,000.00
Sulfuric Acid	8	ea	\$108.00	\$864.00
Methyl Alcohol	5	ea	\$220.00	\$1,100.00
50-lb Bag of Soda Ash	8	ea	\$23.00	\$184.00
50-lb Bag of Baking Soda	10	ea	\$30.00	\$300.00
<b>Total</b>				<b>\$9,448.00</b>

Table 23. Cost of mechanical delinting cottonseed for breeding program including cost of equipment needed and the labor cost for one employee.

<b>Cost</b>	<b>Quantity</b>	<b>Unit</b>	<b>Price</b>	<b>Extended Price</b>
Labor for 1 worker	200	hr	\$8.75	\$1,750.00
Mechanical Delinter	1	ea	\$25,000.00	\$25,000.00
Air Compressor and Accessories	1	ea	\$850.00	\$850.00
<b>Total</b>				<b>\$27,600.00</b>

Comparing the two different methods based on one year of delinting, mechanically delinting is about three times as high as acid delinting. This is a large difference, but the initial cost of the mechanical delinter would be depreciated after several years as the labor cost is the only recurring annual expense. In comparison, acid delinting requires purchasing chemicals every year and a higher labor cost. Other costs such as building requirements, miscellaneous equipment needed for acid delinting, the price of water that is used for acid delinting and proper disposal of effluent produced by acid delinting were not included in the cost analysis. Different methods to dispose of the effluent are used in cotton breeding programs, which included the addition of evaporation tanks that are essentially open-air troughs that would be filled with the effluent and left to evaporate. Additional space would be required for the evaporation tanks as

well was a physical barrier for safety precautions. Another option for effluent removal would be a sewage line connected to a municipal sewer main. This addition would require daily or weekly water samples depending on the municipality. Electricity use for one mechanical delinting unit is not included in the cost analysis as the power required would not differ between the different delinting methods. Acid delinting requires additional power to operate a ventilation system to expel noxious fumes and after the seed is delinted a seed dryer is needed to dry the delinted seed quickly. The electrical power required would increase as the number of mechanical delinters being utilized increased.

#### 5.9 Additional Observations of Note

In 2017, the delinter was used to delint two early generation nurseries. This was done to see how practical it is to use the delinter for breeding material. To minimize the amount of cleaning and seed carry-over the samples were put in order by relatedness of germplasm. The nursery was delinted in this manner to minimize seed carry-over and a small of seed carry-over was tolerated since individual plant selections were going to be selected. The nurseries had a total of about 400 samples. The delinting took two working weeks to complete. Two student workers were instructed in the proper use of the delinter and within one hour they were able to grasp the instructions and capable of delinting without problems or direct supervision. This is important because a mechanical delinter that is too complicated to operate would reduce efficiency in a program and increase the likelihood of cross-contamination of seed lots. The two undergraduate workers had acid delinted for two years and said they preferred the mechanical delinting process over acid delinting.

The delinter used for this study is an early prototype and changes have been made to newer models. Improvements addressed design flaws and improved the usability of the machine.

One example of such an improvement was the enhanced design of the frame that holds the drum in place while delinting.

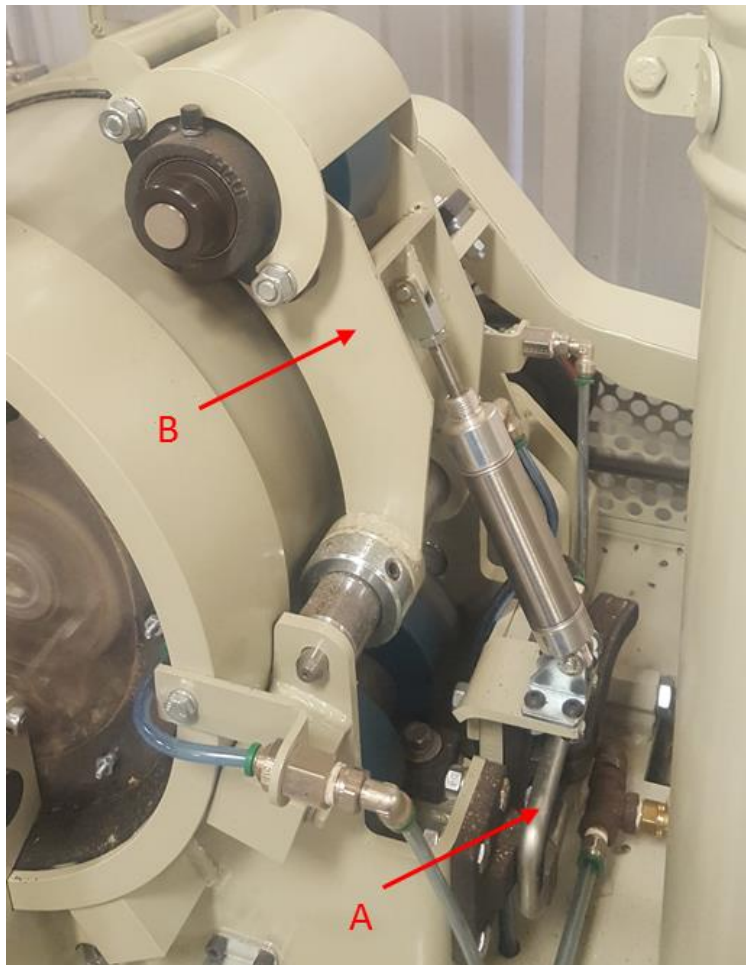


Figure 21. New mechanical cottonseed delinter model frame modification showing a pneumatic roller arm and front face-plate clasp.

Instead of the front face-plate being held in place by four bolts, two clasps were attached to the frame (Figure 21.A). The addition of the clasps reduces the amount of time required to remove the front face-plate because the operator does not have to remove the bolts. It also removes the need for a majority of the tools required to operate the delinter. The placement of the top rollers has been changed as well. The top rollers have been placed on top of hydraulic arms that open and close when a switch is activated on the delinter control panel (Figure 21.B). The addition of

the hydraulic arms makes removing and replacing the drum easier. The design of the linter removal duct has also been modified to address excessive trash buildup. The newer models have replaced the trapezoidal section of the linter removal duct with a straight piece of square tubing. The outlet from the linter removal duct has also been widened to allow larger portions of removed linters to be removed.

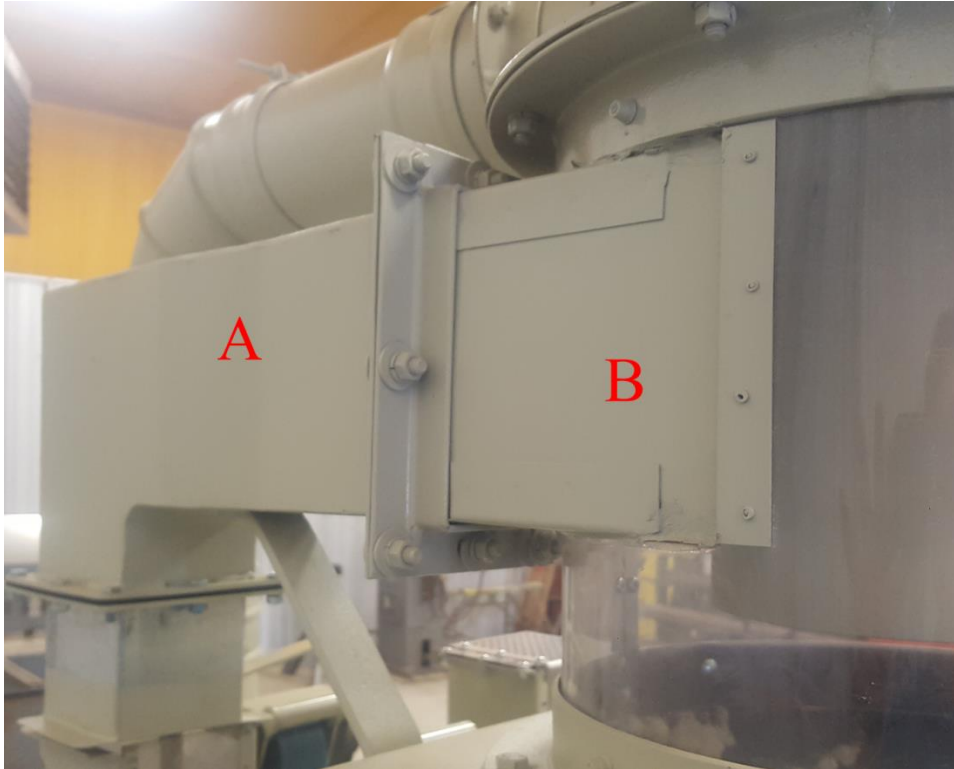


Figure 22. New mechanical cottonseed delinter model lint removal duct modification.

The delinter that was used for testing arrived with many manufacturing flaws that have been addressed in newer models. One flaw that was resolved was the lack of durability of the Lexan face plates which were attached using rivets. The rivets on the face plates tended to pop out after prolonged periods of delinting. To resolve this issue, the rivets were removed and replaced by screws with the pilot holes being counter sunk into the face-plate making the face-plate smooth with no screw protruding.

The delinter also came equipped with a guard covering the pulleys and belts. Whenever the guard was on the delinter it would impede the movement of the pulley and belts rendering the delinter non-operational. Redesigning the guard would resolve this issue as running the delinter without the guard would make using the machine unsafe. When it was decided that it was time to perform maintenance on the delinter and the bearings of the delinter were being greased, it was found that four of the twelve bearings could not be greased because the grease gun insert was facing the wrong way making it difficult if not impossible to attach the gun. This error is not necessarily a design flaw but more of a manufacturing and assembly mishap which should not factor into the overall functionality assessment of the delinter.

## 6. CONCLUSION

The data show that the mechanical brush delinter can delint cottonseed if given enough time. The germination tests and the field germination calculations suggested that the germination percent of the mechanically delinted seed was not as high as acid delinted seed but was in an acceptable range. The mechanical delinter successfully delints seed, but to be used in a breeding program it needs improvements such as the elimination of seed carry-over between samples, improved speed, and reduction of seed borne diseases.

The seed carry-over experiment found carry-over for many samples in both treatments. This same experiment determined the mean number of okra leaf plants is low, but the occurrence of okra leaf plant in the normal leaf plots is a problem that needs attention. The mechanical brush delinter was less efficient than acid, initially taking an average of 4.75 min/sample. The cantilever configuration experiment found that design and configuration of the cantilever brushes affect the delinting time, and it was determined that having both stacked brushes installed decreased delinting time to 2.66 min/sample. The new brush configuration reduced delinting time and design changes to the drum assembly have decreased the amount of time that is required to disassemble and reassemble the delinter for cleaning.

Mechanically delinting seed will also not remove Xcm (bacterial blight). Even though Xcm is still present on the seed coat, the mechanical brush delint can still be an alternative to acid delinting. Neither method would remove Xcm when inside cotton seed. Caution should be used if the seed being delinted came from a location infected with the pathogens. The removal of potential pathogens could be achieved by alternative methods. Additional research would be needed to develop a process to sterilize the seed coat. This could be achieved by the addition of an antibacterial seed treatment, but it is unclear if this is a viable option and research would have

to be conducted to investigate the feasibility or other possible concerns attributed to the additional treatment.

The variable seed size experiment indicated that the delinting time is not well-correlated with seed index. It does indicate that additional genotypic factors such as attachment force may influence delinting time. Overall, the mechanical delinter can be a valuable tool for breeders due to ease of use, safety, effluent reduction and potential linter value. The linters removed in mechanical delinting are valuable as a source of cellulose in the production of plastics and other products, but the amount of linters that a breeding program would produce from mechanical delinting would have a miniscule value. A large mechanical brush delinter would produce large enough quantities of linters to be worthy of marketing. With further improvement to the design, mechanical delinting may become an alternative method of delinting cottonseed for breeding programs.

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